Plant species composition and biofuel yields of conservation grasslands

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Abstract. Marginal croplands, such as those in the Conservation Reserve Program (CRP), have been suggested as a source of biomass for biofuel production. However, little is known about the composition of plant species on these conservation grasslands or their potential for ethanol production. Our objective was to assess the potential of CRP and other conservation grasslands for biofuel production, describing the relationships of plant species richness and tall native C_4 prairie grass abundance with plant chemical composition and the resulting potential ethanol yield. We determined plant species composition and diversity at multiple scales with the modified Whittaker plot technique, aboveground biomass, plant chemical composition, and potential ethanol yield at 34 sites across the major ecological regions of the northeastern USA. Conservation grasslands with higher numbers of plant species had lower biomass yields and a lower ethanol yield per unit biomass compared with sites with fewer species. Thus, biofuel yield per unit land area decreased by 77% as plant species richness increased from 3 to 12.8 species per m^2. We found that, as tall native C_4 prairie grass abundance increased from 1.7% to 81.6%, the number of plant species decreased and aboveground biomass per unit land area and ethanol yield per unit biomass increased resulting in a 500% increased biofuel yield per unit land area. Plant species richness and composition are key determinants of biomass and ethanol yields from conservation grasslands and have implications for low-input high-diversity systems. Designing systems to include a large proportion of species with undesirable fermentation characteristics could reduce ethanol yields.

Key words: biodiversity; biofuel; C_4 grass; conservation reserve program; CRP; ethanol; grasslands; plant diversity; species richness; switchgrass; warm-season grass.

INTRODUCTION

Conservation grasslands serve to reduce soil erosion (Zheng et al. 2004), sequester carbon in the soil (Baer et al. 2002, McLauchlan et al. 2006), improve water quality (Lant et al. 2005), and enhance the wildlife habitat value of the landscape (Murray et al. 2003, Veech 2006). Land set aside for conservation purposes, such as in the Conservation Reserve Program (CRP), has been suggested as a readily available biomass feedstock resource in the United States (National Research Council 2000, De La Torre Ugarte et al. 2003). About 6.8 million of the 12.1 million ha of CRP land (1998 data) may be available for biomass feedstock production (De La Torre Ugarte et al. 2003). Developing alternative and complementary uses for CRP land, such as biofuel production, would provide an economic incentive to keep this land in perennial grass cover and maintain the environmental benefits of the CRP program.

Little is known about the plant species composition or richness, abundance of native vs. nonnative species, aboveground biomass production, or potential biofuel yields on CRP and other conservation grasslands (Baer et al. 2002). Plant community composition and biomass productivity are influenced by many factors including site productivity (Michalet et al. 2006), soil nutrient status (Gough et al. 2000), history of community assembly (Fukami and Morin 2003), latitude (Partel et al. 2007), and management history (Collins et al. 1998). Plant diversity has been suggested as a way to maximize sustainable biomass production of prairies on degraded lands for biofuel production (Tilman et al. 2006).

Ethanol yields vary with plant species resulting from differences in plant cell wall structural chemistry affecting both the carbohydrate composition and efficiency of enzymatic hydrolysis (Dien et al. 2006). Although there is little information on how different plant species affect ethanol yield (Dien et al. 2006), there are ample data demonstrating that plant composition affects forage quality for animals (Fales and Fritz 2007), but limited information is available on the effect of plant diversity (Tracy and Faulkner 2006). Our objective was

to assess the potential of CRP and other conservation grasslands for biofuel production, describing the relationships of plant species richness and tall native C_4 prairie grass abundance with plant chemical composition and the resulting potential ethanol yield.

METHODS

The majority of CRP (Conservation Reserve Program) and CREP (Conservation Reserve Enhancement Program) lands are planted in cool-season (C_3) grasses in the northeastern United States. We focused our study on warm-season (C_4) grass sites because C_4 grasses have been emphasized as biomass crops (Sanderson et al. 2004). We included CRP, CREP, WHIP (Wildlife Habitat Incentives Program), Partners for Wildlife, National Wildlife Refuges, State Parks, Wildlife Management Areas, Pennsylvania State Game Lands, mine land reclamation sites, and other grasslands in the northeast region of the United States. Most sites had a previous land use history of either row crops or hay/pasture. We worked with resource management professionals in several state and federal agencies to identify and obtain permissions to sample individual sites. We chose 34 warm-season grassland sites in New York, Pennsylvania, New Jersey, Maryland, and Virginia and sampled them during late August through mid October in 2002 and 2003 when plants were fully mature (late seed set or beyond) but before first frost. Six sites were sampled both years. Sampling locations covered the major ecological regions of the northeastern United States (Fig. 1; Bailey 1995).

We used the modified Whittaker plot (Stohlgren et al. 1995, 1998) to quantify plant species richness and composition within each conservation grassland. Species richness was recorded at four scales (1, 10, 100, and 1000 m²) with this method. The outer 1000-m² plot (50 × 20 m) contains one 100-m² plot, two 10-m² plots, and 10 1-m² plots. We compiled complete lists of the species present in all plots at all scales and visually estimated plant species cover in each of the 10 1-m² plots. Plant species nomenclature, functional group, and U.S. nativity were as defined on the National PLANTS Database (*available online*).[5] Aboveground biomass and soil samples, management history, initial species seeded, and landscape details were obtained from each location along with weather data. Ten 1-m² plots, at the four corners and adjacent to the six 1-m² plots, surrounding the outside of each Whittaker plot were harvested to determine the amount of standing biomass. The plots were harvested at a 10-cm stubble height and the biomass was dried at 55°C. The dried biomass samples were ground first in a hammer mill and then ground to pass a 1-mm screen of a Wiley mill (Thomas Scientific, Swedesboro, New Jersey, USA).
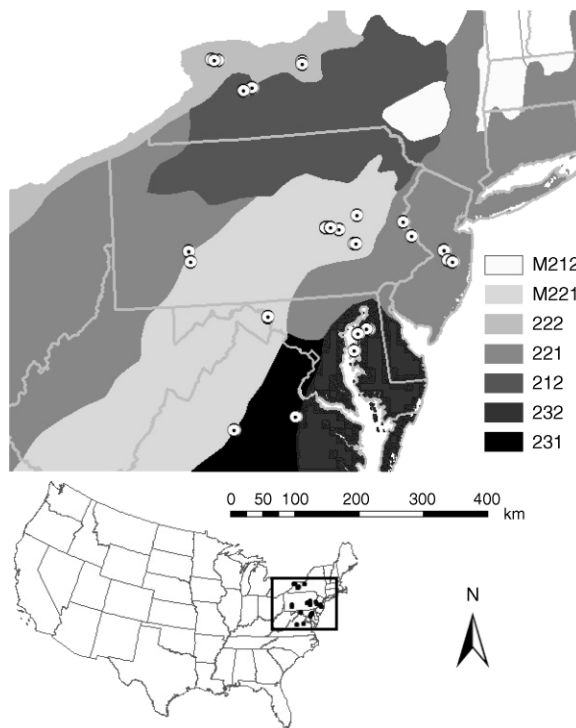

FIG. 1. Location of conservation grasslands included in the survey. Regional ecoregion provinces (Bailey 1995) include: 212 (Laurentian Mixed Forest Province), M212 (Adirondack–New England Mixed Forest–Coniferous Forest–Alpine Meadow Province), 221 (Eastern Broadleaf Forest [Oceanic] Province), 222 (Eastern Broadleaf Forest (Continental) Province), M221 (Central Appalachian Broadleaf Forest–Coniferous Forest–Meadow Province), 231 (Southeastern Mixed Forest Province), and 232 (Outer Coastal Plain Mixed Forest Province). See ⟨http://www.fs.fed.us/colorimagemap/ecoreg1_provinces.html⟩ for complete ecoregion descriptions.

The biomass samples were analyzed for cellulose, hemicellulose, and lignin. Samples were scanned on a near-infrared reflectance spectrophotometer (NIRS, Model 6500; FOSS NIRSystems, Silver Spring, Maryland, USA). A calibration set of 66 samples for neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were chosen by cluster analysis of the reflectance data (Shenk and Westerhaus 1991). Calibration samples were analyzed in duplicate for NDF and ADF with the ANKOM Fiber Analyzer (ANKOM Technology Corporation, Fairport, New York, USA) following Vogel et al. (1999) and the ANKOM ADL procedure (procedure *available online*).[6] Laboratory means were used to develop calibration equations by partial least squares regression (Shenk and Westerhaus 1991). Cellulose concentration was estimated as ADF minus ADL and hemicellulose as the difference between NDF and ADF values.

[5] ⟨http://plants.usda.gov⟩

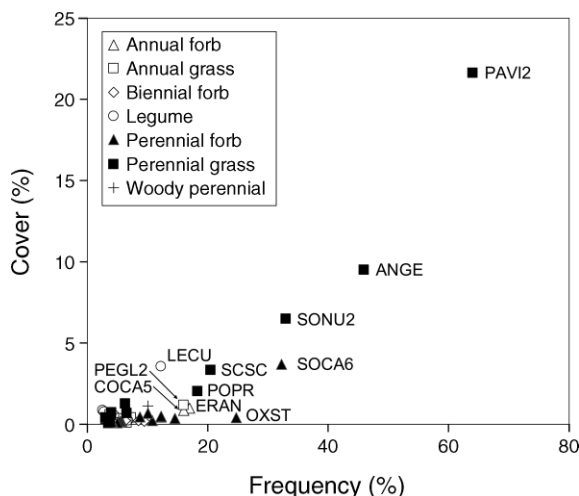[6] ⟨http://www.ankom.com/09_procedures/ADL%20in%20beakers.pdf⟩

FIG. 2. Cover of the dominant species found on the conservation grasslands surveyed in the northeastern United States using the modified Whittaker plot technique. Of the 285 plant species identified across all sites, the 60 with the highest importance value are plotted in the graph, accounting for over 90% of the plant cover. The most dominant species labeled in the graph accounted for more than 70% of the plant cover, and almost 90% of that cover was from native plants. Cover values indicate the percentage of the total mean cover at all sites and in the 10 1-m^2 quadrats at each site. Frequency indicates the percentage of 1-m^2 quadrats in which the species were found. Species identifications are: PAVI2 (*Panicum virgatum* L.), ANGE (*Andropogon gerardii* Vitman), SONU2 (*Sorghastrum nutans* (L.) Nash), SOCA6 (*Solidago canadensis* L.), OXST (*Oxalis stricta* L.), SCSC (*Schizachyrium scoparium* (Michx.) Nash), POPR (*Poa pratensis* L.), ERAN (*Erigeron annuus* (L.) Pers.), PEGL2 (*Pennisetum glaucum* (L.) R. Br.), COCA5 (*Conyza canadensis* (L.) Cronq.), and LECU (*Lespedeza cuneata* (Dum.-Cours.) G. Don).

We used two methods to estimate the bioconversion potential of the biomass materials. The first, a potential ethanol yield, was calculated as 75% of the sum of cellulose and hemicellulose using the "theoretical ethanol yield calculator" (*available online*),[7] a reasonable estimate of current commercial technology based on Dien et al. (2006). This calculation is based on the proportion of hexose and pentose sugars in the biomass and assumes that pretreatments and acid or chemical hydrolysis will make these sugars freely available for fermentation and that improved yeast strains will completely ferment both the hexoses and pentose monomers. The second method, in vitro gas production, relies on anaerobic fermentation of the biomass by a mixed microbial inoculum from the bovine rumen without pretreatment of the biomass beyond simple grinding and without addition of exogenous enzymes. This method provides an indication of fermentability that might be expected from consolidated bioprocessing, an alternative cellulosic ethanol platform that uses

[7] ⟨http://www.eere.energy.gov/biomass/ethanol_yield_calculator.html⟩

anaerobic bacteria that produce their own cellulose- and hemicellulose hydrolases and that ferment the resulting sugars to ethanol and other products (Lynd et al. 2002). The in vitro gas production method provides a surrogate measure of the fermentability of cellulosic biomass to ethanol, suitable for screening large numbers of biomass samples (Weimer et al. 2005). In vitro gas production analyses were conducted as described previously (Weimer et al. 2005) on composite samples for each site, and gas production data across duplicate 96-h runs were normalized using data from the fermentation of alfalfa fiber standards included in each run.

We calculated Pearson correlation coefficients (r) between species richness or tall C_4 prairie grass cover and chemical composition or potential ethanol yield with the PROC CORR procedure in SAS (SAS Institute 2002).

RESULTS

The tall C_4 prairie grasses (*Panicum virgatum* L, *Andropogon gerardii* Vitman, and *Sorghastrum nutans* (L.) Nash) were the most dominant species (Fig. 2) and the number of plant species decreased as the cover of these C_4 grasses increased (Fig. 3; $r = -0.40$, $P = 0.0105$). Native species accounted for most of the 285 plant species we identified across all the conservation grasslands.

The concentration of lignin, cellulose, and hemicellulose varied with plant species composition and species richness, which ultimately affected the potential ethanol yield of the sites. Lignin concentration did not change as plant species richness increased (Fig. 4a; $P = 0.9936$).
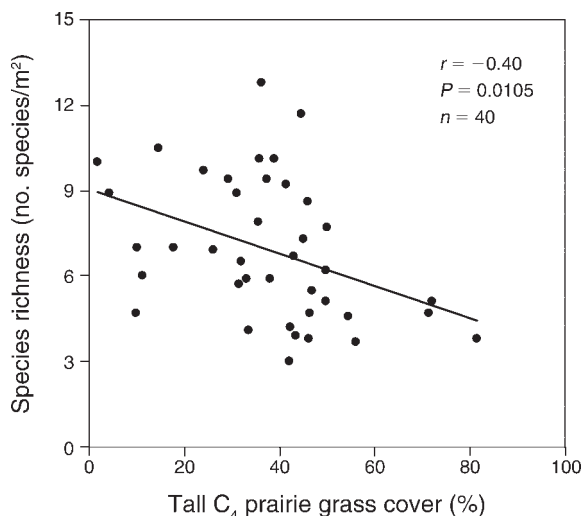

FIG. 3. Correlation of species richness with tall C_4 prairie grass species cover in 1-m^2 quadrats. Tall C_4 prairie grass species included in cover measurement were *Panicum virgatum*, *Andropogon gerardii*, and *Sorghastrum nutans*. The number of observations ($n = 40$) for species richness and tall C_4 prairie grass cover were means of 10 1-m^2 sampling points.
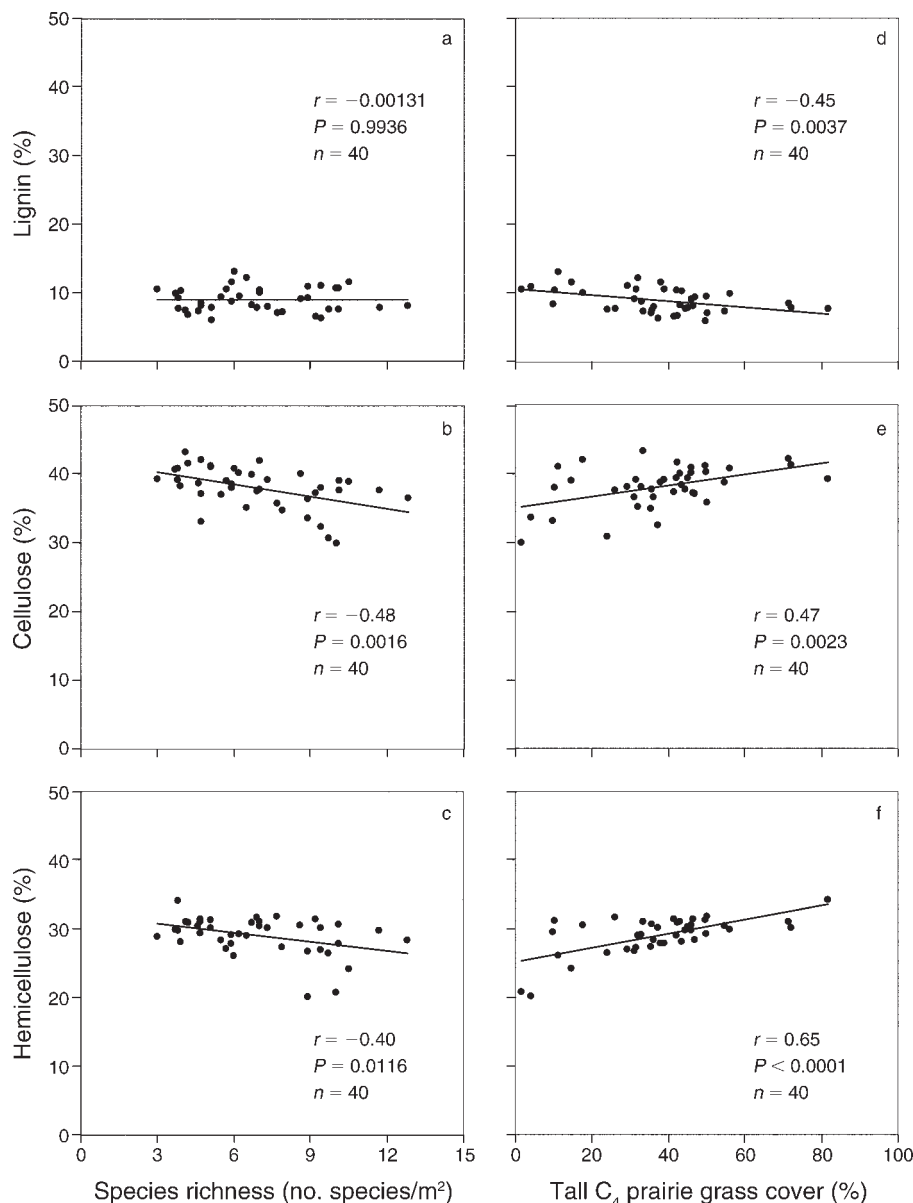
FIG. 4. Correlation of lignin, cellulose, and hemicellulose concentrations with species richness and tall C_4 prairie grass species cover in 1-m^2 quadrats. Tall C_4 prairie grass species included in cover measurement were *Panicum virgatum*, *Andropogon gerardii*, and *Sorghastrum nutans*. The number of observations ($n = 40$) for chemical composition, species richness, and tall C_4 prairie grass cover were means of 10 1-m^2 sampling points.

However, both cellulose (Fig. 4b; $r = -0.48$, $P = 0.0016$) and hemicellulose (Fig. 4c; $r = -0.40$, $P = 0.0116$) were negatively correlated with plant species richness. The decrease in cellulose and hemicellulose concentration with greater species richness reduced the potential ethanol yield (on a unit biomass basis; Fig. 5a; $r = -0.51$, $P = 0.0007$). Because aboveground biomass yield decreased with greater species richness (P. R. Adler, M. A. Sanderson, and S. C. Goslee, *unpublished manuscript*) potential ethanol yield on a unit land area basis was also negatively correlated with plant species richness (Fig. 5b; $r = -0.53$, $P = 0.0005$). Lignin

concentration was negatively correlated with the cover of tall C_4 prairie grasses (Fig. 4d; $r = -0.45$, $P = 0.0037$). Both cellulose (Fig. 4e; $r = 0.47$, $P = 0.0023$) and hemicellulose (Fig. 4f; $r = 0.65$, $P < 0.0001$) concentrations were positively correlated with tall C_4 prairie grass cover. The increase in cellulose and hemicellulose concentrations with tall C_4 prairie grass cover resulted in an increased potential ethanol yield on a mass basis (Fig. 5c; $r = 0.65$, $P < 0.0001$). Because aboveground biomass yield increased with the cover of tall C_4 prairie grasses (P. R. Adler, M. A. Sanderson, and S. C. Goslee, *unpublished manuscript*) potential ethanol yield on a unit
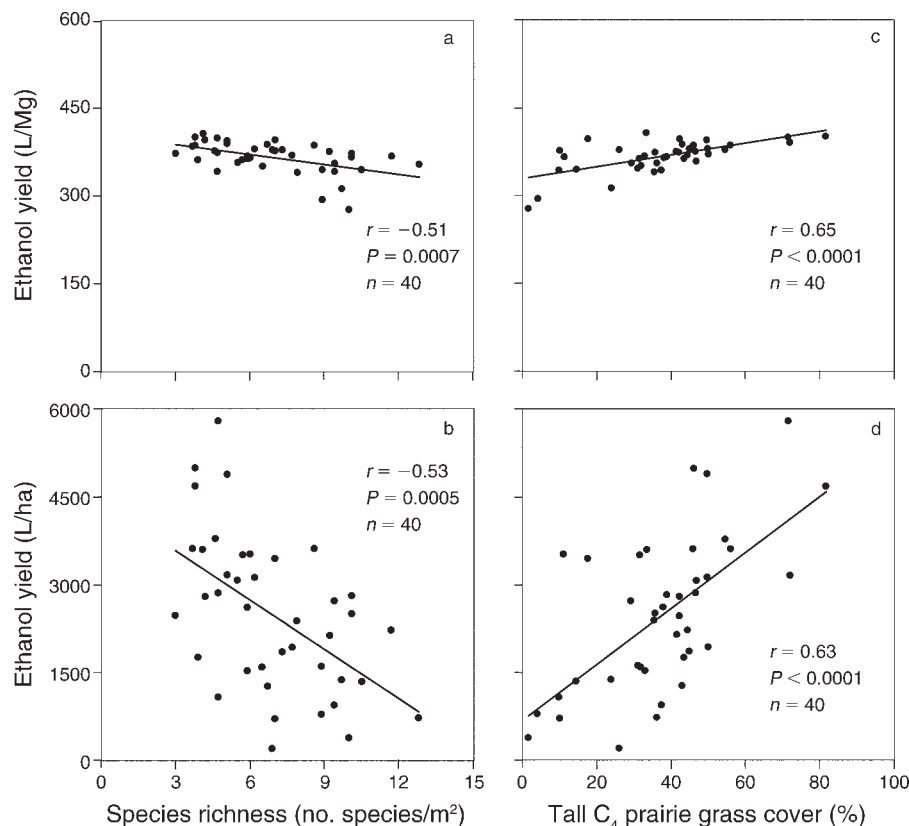
FIG. 5. Correlation of ethanol yields with species richness and tall C_4 prairie grass species cover in 1-m^2 quadrats expressed on a unit-biomass and land-area basis. Tall C_4 prairie grass species included in cover measurement were *Panicum virgatum*, *Andropogon gerardii*, and *Sorghastrum nutans*. The number of observations ($n = 40$) for potential ethanol yield, species richness, and tall C_4 prairie grass cover were means of 10 1-m^2 sampling points.

land area basis was also positively correlated with tall C_4 prairie grass cover (Fig. 5d; $r = 0.63$, $P < 0.0001$).

Potential ethanol yield estimated by the in vitro gas production and chemical composition methods reflect different conversion processes. The in vitro gas production method includes the potential inhibitory effects from lignin, which may not be present in appropriately pretreated biomass. By contrast, ethanol yields estimated by chemical composition assume there are no inhibitory effects of either residual lignin or of side-products of pretreatment. Therefore it is not surprising that results comparing the two methods are not completely consistent. The in vitro gas production method was not related to species richness (Fig. 6a; $r = 0.11$, $P > 0.5$); however, as with ethanol yield predicted from chemical composition, gas production was positively correlated with tall C_4 prairie grass cover (Fig. 6b; $r = 0.37$, $P = 0.0192$).

DISCUSSION

Plant species composition affected the concentration of lignin, cellulose, and hemicellulose in the biomass (Fig. 4) and significantly altered the potential ethanol yield on these conservation grasslands. From linear regression analysis of data in Fig. 5, the potential ethanol yield decreased 14% per unit biomass and 77% per unit land area as plant species richness increased from 3 to 12.8 species per m^2. The reduction in potential ethanol yield was magnified because aboveground biomass yield per unit area also decreased with greater plant species richness, leading to a decrease in potential ethanol yield per unit land area. Potential ethanol yield, however, increased 24% per unit biomass and more than 500% per unit land area as the cover of tall C_4 prairie grasses increased from 1.7% to 81.6%. Results from the alternative method of assessing biomass fermentability, in vitro fermentation, did not indicate a response to species richness, but did reveal a positive relationship between fermentability and cover of tall C_4 prairie grasses. The effect of plant species composition on potential ethanol yield needs to be considered when describing ethanol production from diverse grasslands.

Research on the chemical composition of biomass has focused on individual forage and crop species such as switchgrass, reed canarygrass, alfalfa, and maize (Dien et al. 2006) with little work on mixed-species biomass (Tracy and Faulkner 2006). Detailed analyses of cell wall composition and ethanol yields of switchgrass, reed

canarygrass, and alfalfa revealed significant species differences (Dien et al. 2006). Lignin, cellulose, and hemicellulose compositions were within 5% between reed canarygrass and switchgrass harvested at maturity. However, lignin and cellulose were higher and hemicellulose lower in alfalfa stems compared with reed canarygrass and switchgrass. In our study, many nonagricultural plants contributed significantly to the biomass on the sites with greater plant species richness. Most of the nonagricultural species were forbs and might be expected to have very different biomass composition compared with the C_4 grasses and could reduce the ethanol production potential. We need to know more about the chemical composition of these species particularly in the light of interest in low-input high diversity (LIHD) grasslands for biomass production (Tilman et al. 2006).

Plant developmental morphology also affects plant cell wall composition and consequently potential ethanol yields (Sanderson and Wolf 1995, Casler and Boe 2003, Dien et al. 2006). Therefore, time of biomass harvest will also affect potential ethanol yields. Lignin, cellulose, and hemicellulose concentrations in C_4 grasses generally increase with plant maturity (Sanderson and Wolf 1995). Concentrations of cell wall components also increase across seasonal harvest times from summer to fall (Casler and Boe 2003, Dien et al. 2006) and spring after over wintering in the field (Adler et al. 2006). The C_4 grasses were mature (past seed set) by the time they were harvested in our study and the lignin, cellulose, and hemicellulose concentrations would be expected to be in between that expected for a late summer or fall harvest.

Competitively dominant species such as the tall C_4 prairie grasses are critical to the function of biomass production in native (Smith and Knapp 2003) and restored conservation grasslands (Baer et al. 2002) often accounting for >70% of the biomass. Croplands restored to prairie typically have fewer plant species than undisturbed prairies (Baer et al. 2004), a challenge to be faced with establishing highly diverse prairie ecosystems whether for conservation grasslands such as CRP or a future biofuel feedstock. In native prairies, the tall C_4 prairie grasses such as big bluestem and switchgrass, dominate different landscape positions, with switchgrass being a more mesic grass (Knapp 1985) and consequently, planting mixtures of tall C_4 prairie grasses may be beneficial to exploit these differences among species in site adaptability.

A number of factors could lead to the large range of native C_4 grass cover observed on these conservation grasslands. During the establishment of the C_4 grasses lack of a firm seed bed, planting seed too deeply, lack of rainfall during establishment, and plant competition from the seed bank or surviving perennial species associated with previous land history can all influence community assembly. According to records provided by the land managers, fewer than 10 species were planted at each site; however, we identified many species from
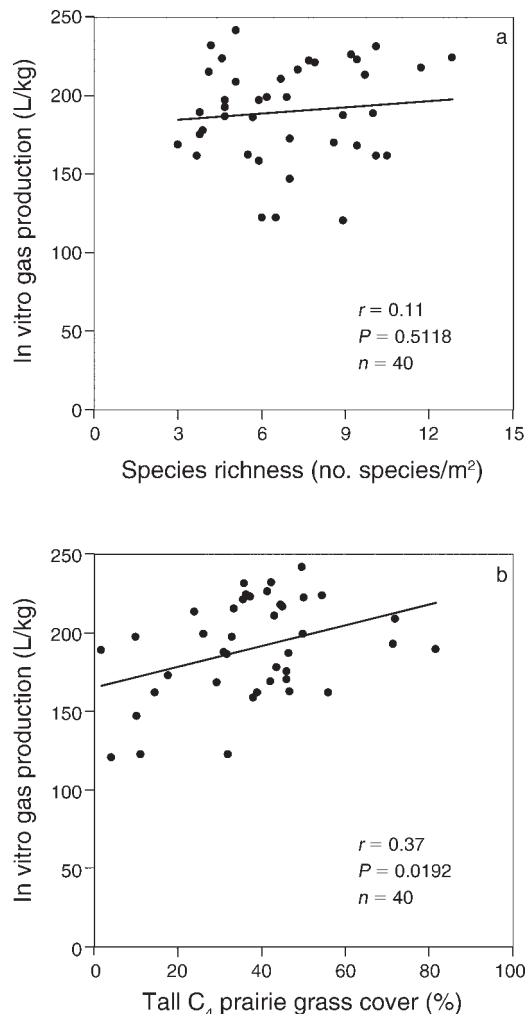

FIG. 6. Correlation of in vitro gas yields with species richness and tall C_4 prairie grass species cover in 1-m^2 quadrats expressed on a unit biomass basis. Tall C_4 prairie grass species included in cover measurement were *Panicum virgatum*, *Andropogon gerardii*, and *Sorghastrum nutans*. The number of observations ($n = 40$) for in vitro gas yields, species richness, and tall C_4 prairie grass cover were means of 10 1-m^2 sampling points.

several functional groups (Fig. 2). The total plant species richness of the sites ranged from 12 to 60 species with a mean of 34 per 0.1 ha. The majority of these plant species were native and accounted for almost 80% of the plant cover. The most dominant plant species are specifically identified in Fig. 2; they accounted for more than 70% of the plant cover and almost 90% of that cover was from native plants. The three most dominant plant species were the tall native C_4 prairie grasses. The highly competitive native C_4 grasses (Fargione and Tilman 2005) reduced the number of plant species on the conservation grasslands (Fig. 3). The characteristic of having a higher use efficiency of nutrients would allow native C_4 grasses to be able to produce more biomass with the limited resources available at these sites.

Implications of managing conservation grasslands for biofuel production

The potential ethanol yield ranged from 200 to almost 5800 L/ha in this survey of 34 conservation grasslands in the northeastern United States. Conservation grasslands dominated by tall native C_4 prairie grasses had more aboveground biomass, fewer plant species, higher ethanol yield per unit biomass, and consequently a higher ethanol yield per unit land area than other sites. Management to increase the cover of tall C_4 prairie grasses on low productivity sites may be necessary to increase biomass and biofuel yields. Although 285 plant species were identified on the grasslands in this survey, native species were dominant. Tall native C_4 prairie grasses are highly competitive and efficient users of limited resources, allowing them to produce more biomass than other species. C_4 grasses and legumes have been shown to be key functional groups for biomass productivity (Fornara and Tilman 2008), producing equivalent biomass yields compared to sites with higher numbers of species. Consequently, management to enhance legume abundance and N_2 fixation may be critical to yield with reduced inputs. Our data suggest that the species composition of plant mixtures used for low-input high-diversity systems (Tilman et al. 2006) affects both biomass production and chemical composition. Including a large proportion of species with undesirable fermentation characteristics could reduce ethanol yields.

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